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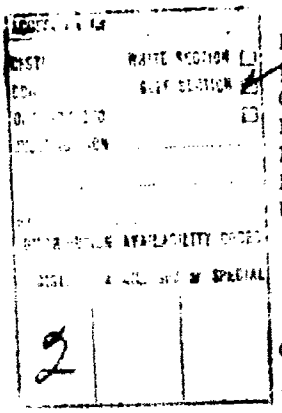
TECHNICAL MANUSCRIPT 567

ASSAY OF CHIKUNGUNYA VIRUS IN CELL MONOLAYERS BY IMMUNOFLUORESCENCE

Nicholas Hahon
William A. Hankins

DECEMBER 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland



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ASSAY OF CHIKUNGUNYA VIRUS IN CELL MONOLAYERS BY IMMUNOFLUORESCENCE

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December 1969

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ABSTRACT

Chikungunya virus was assayed quantitatively by counting immunofluorescent foci after infection of BHK21/C13 cell monolayers. The speed and efficiency of virus attachment to cells were markedly enhanced when augmented by centrifugal force. By this procedure, a proportionality was obtained between the number of immunofluorescent foci and the volume of inoculum. Virus penetration into cells was linear and complete within 15 minutes at 35 C. From observations on the sequential development of viral antigen within cells and immunofluorescent focus counts, foci of infected cells may be enumerated as early as 16 hours after inoculation of cell monolayers. A linear function was demonstrated between immunofluorescent focus counts and relative virus concentration. The immunofluorescent assay was comparable in sensitivity to but more precise and rapid than virus assays based on the intracerebral inoculation of suckling mice or on plaque counting. By the immunofluorescence procedure, the 50% neutralizing end point of antiviral serum was rapidly and quantitatively determined.

I. INTRODUCTION*

Quantitative assays of virus infectivity based on enumeration of cells containing immunofluorescent viral antigens have been established for agents representative of almost all major animal virus groups and, recently, the procedure has been successfully employed to assay a plant virus.[1] Of the large number of agents that comprise the different arbovirus groups, however, immunofluorescent assays have been developed for only four viruses: yellow fever,[2] Venezuelan equine encephalomyelitis,[3] Semliki Forest fever,[4] and Rift Valley fever.[5] The feasibility of extending the immunofluorescent technique for the infectivity assessment of another arbovirus, chikungunya, was investigated because it has been demonstrated that cells infected with the virus are amenable to immunofluorescent staining.[6,7] This is a report on the development and evaluation of this technique for the quantitative assay of chikungunya virus.

II. MATERIALS AND METHODS

A. VIRUS STRAIN

The Banganike strain of chikungunya virus[8] used in this study was in the form of a 10% suckling mouse brain suspension. Assayed in suckling mice, the suspension had a titer of $10^{8.6}$ LD_{50}/ml by intracerebral inoculation.

B. CELL LINES AND CULTIVATION

The principal cell line used for assay of the virus was baby hamster kidney (BHK21/C13) obtained from the American Type Culture Collection, Rockville, Md. Morphologically, this cell line consists of elongated fibroblastic cells. Nutrient medium consisted of Earle's minimum essential medium (MEM) supplemented with 1% glutamine (200 mM), 10% tryptose phosphate broth, 10% fetal calf serum, 0.005% neomycin, and 75 µg kanamycin/ml. Maintenance medium was equal parts of nutrient medium and MEM. Cells were cultivated on circular cover slips (15-mm diameter) inserted in flat-bottomed glass vials (19 by 65 mm). A 1-ml cell suspension containing from 10^5 to 3×10^5 cells was introduced onto cover slips, which were then incubated at 35 C for 24 hours or until a complete cell monolayer was formed.

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C. IMMUNOFLUORESCENT VIRUS ASSAY

Determinations were usually made in triplicate. Initially, virus dilutions were prepared in phosphate-buffered saline (PBS) solution, pH 7.1, free of calcium and magnesium ions,[3] but later, heart infusion broth (HIB) was used. After transfer of the cell monolayers on cover slips from glass vials to rotor chamber inserts,[2] 0.2 ml of the virus suspension to be assayed was added to the inserts. The rotor chamber inserts were used because they withstood the high centrifugal force required to sediment the virus. Rotor chamber inserts placed in a swinging-bucket SW 25.1 rotor were centrifuged in a model L Preparative Ultracentrifuge* at 19,642 to 29,432 x g for 15 minutes at 3C C. The residual inoculum was removed after centrifugation, the cover slip cell monolayers were replaced into glass vials, and 1 ml of maintenance medium was then added to each vial. After incubation at 35 C for ½ to 1 hour, the maintenance medium was replaced with 1 ml of a 1/15 dilution of monkey viral antiserum in maintenance medium. The rationale for this procedure is presented in Section III. After further incubation at 35 C for 20 to 22 hours, cover slip cell monolayers were rinsed twice with PBS solution, fixed with cold (-60 C) acetone, and either prepared immediately for immunofluorescent staining and focus counting or stored at -60 C. Fluorescence of viral antigens in fixed cell cultures was not diminished on storage of cell cultures for several weeks.

D. CHIKUNGUNYA ANTISERUM CONJUGATE AND IMMUNOFLUORESCENCE STAINING

Antiviral serum was obtained from rhesus monkeys that had been injected intravenously with 10^7 immunofluorescent focus units (IFU) of virus in 1-ml volume. Animals were injected twice during a period of 1 month. Two weeks after the last injection, the animals were bled. Antiserum was conjugated with fluorescein isothiocyanate by the method of Riggs et al.[9] Conjugated globulin was passed through a column of Sephadex G-50** to remove unbound dye. To reduce nonspecific fluorescence, 5 ml of conjugated globulin were diluted with an equal volume of PBS solution and adsorbed twice with 200 mg of acetone-dried mouse liver powder in accordance with the procedure of Coons and Kaplan.[10]

The direct fluorescent antibody method was used to demonstrate immunofluorescence of viral antigens in infected cells. Fixed cell monolayers were washed once with PBS solution and stained with conjugated globulin for 30 minutes at room temperature. Cover slip cell monolayers were then rinsed in two changes of PBS solution and mounted in a semipermanent medium.[11]

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* Beckman Instruments, Inc., Spinco Division, Palo Alto, Calif.
** Pharmacia Fine Chemicals, Inc., Piscataway, N.J.

E. FLUORESCENCE MICROSCOPY AND FOCUS COUNTING

Cover slip cell cultures were examined with an American Optical microscope equipped with a Fluorolume illuminator (model 645), Corning No. 5849 and Schott BG-12 exciter filters, and an E.K. No. 2A barrier filter. With this optical system at a magnification of 430X, the number of microscopic fields contained in the area of a 15-mm cover slip was 1,064. On occasion, when cell monolayers contained numerous immunofluorescent foci, 200X magnification was employed; the number of fields per cover slip was 226. For each cover slip cell monolayer, 50 microscopic fields were examined for immunofluorescent foci. To calculate the number of IFU of virus per milliliter, the average number of IFU per field was multiplied by the number of fields per cover slip, the reciprocal of the dilution of virus inoculum, and a volume factor (for conversion to milliliters).

F. SUCKLING MOUSE VIRUS ASSAY

One suckling mouse litter was inoculated for each virus dilution; each litter consisted of eight 1- to 2-day-old mice. Appropriate virus dilutions in 0.02-ml volume were inoculated intracerebrally into each mouse. Deaths occurring within 24 hours after inoculation were considered nonspecific. Mice were observed for deaths daily for 10 days. Virus titers were calculated by the Reed and Muench[12] formula and expressed as suckling mouse intracerebral median lethal dose ($SMICLD_{50}$).

G. PLAQUE VIRUS ASSAY

Monolayers of guinea pig lung (GPL), BHK21/C13, or chick embryo fibroblast (CEF) cells grown in 25-cm^2 flasks* were inoculated with 0.1 ml of appropriate virus dilutions and then incubated at 37 C for 1 hour. Five milliliters of overlay medium was added to each flask. This medium consisted of basal medium Eagle (Earle's base), 2% fetal calf serum, 2% Noble agar,** 100 µg of DEAE-dextran,*** 100 units of streptomycin, and 100 µg of penicillin per ml. GPL cell monolayers were incubated at 33 C for 72 hours; BHK21/C13 and CEF cells were incubated at 33 C for 48 hours. The second overlay was then applied; it was similar to the first except that it contained 0.01% neutral red but no DEAE-dextran. After additional incubation of cell monolayers at 33 C from 18 to 24 hours, plaques were counted.

H. DETERMINATION OF VIRUS ATTACHMENT

Virus attachment was measured by observing and recording the disappearance of virus from inoculum after its addition to cell monolayers. Cover slip cell cultures to be subjected to centrifugation or stationary incubation at 35 C received 0.2 ml and 0.4 ml of inoculum, respectively. After designated

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* Falcon Plastics, Los Angeles, Calif.
** Difco Laboratories, Detroit, Mich.
*** Pharmacia Fine Chemicals, Inc., Piscataway, N.J.

incubation intervals, residual inoculum was removed, and the cell monolayers were immediately washed twice with PBS solution. Residual inoculum was then introduced onto fresh cell monolayers to measure unattached virus. For this, the residual inoculum was attached onto cell cultures by centrifugation at 19,642 to 29,432 x g for 15 minutes. Cover slip cell monolayers exposed to initial or residual inocula were then treated in the manner described earlier for assay of virus. The amount of virus that was attached to cells at a given time was expressed as a percentage of the virus input. The latter was the sum of the amounts of attached and residual virus.

I. DETERMINATION OF VIRUS PENETRATION

Virus penetration into cells was measured by the insensitivity of attached virus to antiserum. Cell cultures were washed twice with PBS solution, overlaid at designated times with 1 ml of a 1/15 dilution of viral antiserum, and then incubated at 35 C for 20 hours. The quantity of virus that penetrated into cells at a given time was expressed as a percentage of the input virus.

J. CALCULATION OF ATTACHMENT AND PENETRATION CONSTANTS

The attachment and penetration rate constants (K) were calculated from the relationship $2.3 \log (V_0/V_t)/nt$, where V_0 = the input virus concentration, V_t = unattached or unpenetrated virus at time t, and n = the number of cells per cubic centimeter determined by resuspension of cover slip cell cultures after trypsin treatment and enumeration of cells in a hemocytometer.

III. RESULTS

A. VIRUS ATTACHMENT

The rate of chikungunya virus attachment onto cover slip BHK21/C13 cell monolayers was determined after centrifugation and after stationary incubation (35 C). Virus inputs for the former and the latter were 740 and 1,480 IFU, respectively. The concentration of cells per cover slip culture was 4.0×10^5/ml. Aided by centrifugal force, approximately 95% of the virus inoculum was attached within 10 minutes; after stationary incubation for 2 hours only 14% of virus was attached (Fig. 1). The attachment rate constants (K) were 3.4×10^{-7} cm^3 min^{-1} with centrifugation and 2.8×10^{-9} cm^3 min^{-1} with stationary incubation. The former attachment rate constant is comparable to those reported for Venezuelan equine encephalomyelitis (VEE) and Rift Valley fever (RVF) viruses under similar experimental conditions.[3,5]

The efficiency of centrifugation and stationary incubation for infecting cell monolayers from different volumes of inoculum is shown in Table 1. A proportionality between the number of immunofluorescent foci and volume of inoculum was obtained when centrifugation was employed. With stationary incubation, a similar relationship was not evident.

Because previous studies with another group A arbovirus (VEE virus) indicated that the efficiency of virus attachment to cells was markedly influenced by the menstruum used to suspend the virus,[3] this factor was also investigated for attachment of chikungunya virus. Virus was diluted in each of four different suspending media, pH 7.1 to 7.3. Before virus inoculum was added, cell monolayers were washed with their respective test diluent. Virus inoculum was centrifuged onto cell cultures; then they were treated in accordance with the described assay procedure. Results (Table 2) show that the composition of the medium used for attachment of chikungunya virus to cell monolayers did not significantly affect assay values. Slightly more virus appeared to be attached, however, in the presence of HIB.

B. VIRUS PENETRATION

The rate of virus penetration into cells was followed by determining the insensitivity of attached virus to antiviral serum at designated times. A virus input of 168 IFU was introduced onto cell monolayers. They were then treated in the manner described in Section II. Results (Fig. 2) indicated that virus penetration at 35 C proceeded at a linear rate without evidence of an initial lag period. Approximately 50% of attached virus penetrated cells within 7.5 minutes; the process was complete within 15 minutes. The penetration rate constant (K) of 2.3×10^{-7} cm^3 min^{-1} was faster than that noted with VEE and RVF viruses.[3,6]

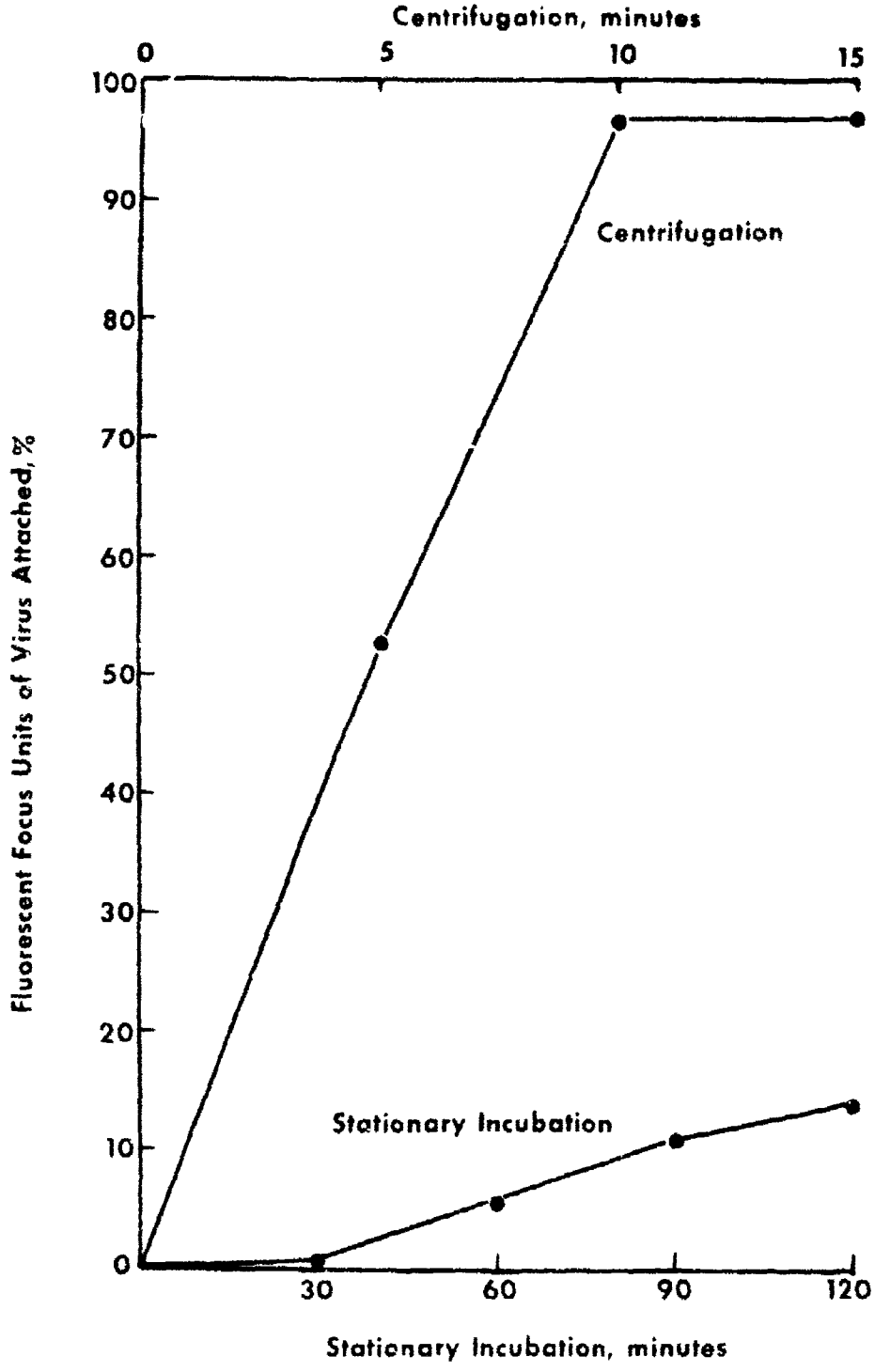

FIGURE 3. Attachment of Chikungunya Virus onto Cover Slip BHK21/C13 Cell Monolayers by Centrifugation (59,682 to 29,537 x g at 25°C) and Stationary Incubation (37°C).

TABLE 1. RELATIONSHIP BETWEEN VOLUME OF INOCULUM AND IMMUNOFLUORESCENT FOCUS UNITS OF CHIKUNGUNYA VIRUS: CENTRIFUGATION VS. STATIONARY INCUBATION

Volume, ml	IFU per 50 Microscopic Fields	
	Centrifugation[a]	Stationary Incubation[b]
0.1	33	11
0.2	60	23
0.5	159	19
1.0	332	29

a. Virus inoculum centrifuged at 19,642 to 29,432 x g for 15 minutes onto BHK21/C13 cell monolayers at 25 C.
b. Stationary incubation of virus inoculum at 35 C for 2 hours.

TABLE 2. EFFECT OF VIRUS DILUENT ON ATTACHMENT OF CHIKUNGUNYA VIRUS ONTO BHK21/C13 CELL MONOLAYERS

Virus Diluent	IFU per 50 Microscopic Fields	Virus Titer, IFU/ml
PBS[a]	43	4.2×10^8
PBS + 0.0009 M $CaCl_2$ + 0.0005 M $MgCl_2$	42	4.2×10^8
Cell maintenance medium[b]	42	4.2×10^8
HIB	56	5.8×10^8

a. NaCl (0.1 M) buffered by 0.01 M phosphate buffer.
b. Minimum essential medium, 5% fetal calf serum, and 5% tryptose phosphate broth.

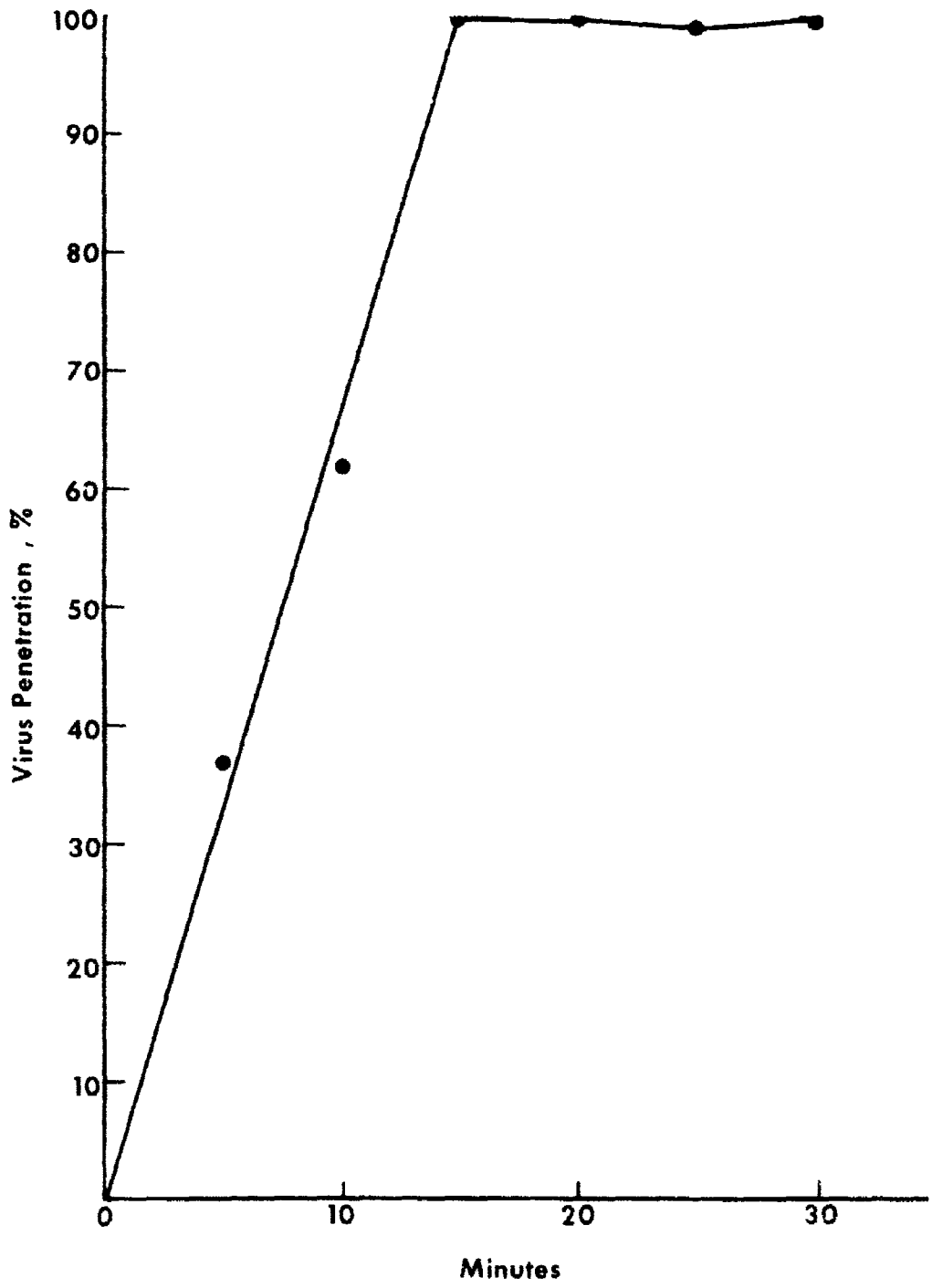

FIGURE 2. Rate of Chikungunya Virus Penetration into BHK21/C13 Cells at 35 C as Measured by Insensitivity of Attached Virus to Antiviral Serum.

Because an antiviral serum overlay is employed in this assay to prevent the extracellular spread of virus from infected cells, this information on the rate of virus penetration is highly relevant. The earliest time that the antiviral serum overlay may be added without neutralizing attached virus was fixed by the results obtained from the preceding experiment. For routine assay of virus, the antiviral serum overlay was added after cell monolayers were incubated at 35 C for ½ to 1 hour following virus attachment.

C. SUSCEPTIBILITY OF CELL LINES

Chikungunya virus was assayed by the immunofluorescence procedure in different cell lines to determine their susceptibility to infection. The cell lines tested were BHK21/C13, BHK21 (short fibroblastic cells obtained from Microbiological Associates, Bethesda, Md.), KB (Eagle), Hep-2, L-929, GPL, and McCoy. Virus was inoculated onto cell monolayers in the usual manner and cell cultures were then incubated in the presence of antiviral serum overlay. Results (Table 3) show that virus assayed in the BHK21/C13 cell line was approximately 1.0 $\log_{10}$ unit higher in titer than that obtained in the BHK21 cell line. In both cell lines, immunofluorescent foci appeared in the presence of antiviral serum; focus counts were the basis of assay values. In the other cell lines, the assay was based on individual infected cell counts because immunofluorescent foci did not appear in the presence of the antiviral serum overlay. The BHK21/C13 cell line was the most susceptible to virus infection of the cell lines tested and was subsequently employed for the standardization of the assay.

TABLE 3. ASSAY OF CHIKUNGUNYA VIRUS IN DIFFERENT CELL LINES

Cell Line	Virus Titer/ml
BHK21/C13	3.1×10^8 IFU
BHK21	2.1×10^7 IFU
L-929	3.3×10^7 CIU[a/]
GPL	1.8×10^7 CIU
McCoy	5.8×10^4 CIU
KB (Eagle)	1.0×10^4 CIU
Hep-2	2.1×10^3 CIU

a. Cell-infecting units of virus.

D. INCUBATION PERIOD

Incubation period is defined as the time interval between virus inoculation and the development of recognizable quantities of fluorescent viral antigens in infected cells. The period was established from sequential observations of infected cell monolayers and immunofluorescent focus counts. Two groups of BHK21/C13 cover slip cell monolayers were inoculated with 2×10^3 IFU in 0.2-ml volume and treated in the manner described for attachment of inoculum. After the prescribed period for virus penetration into cells, antiviral serum overlay was added to one group of cell monolayers; the other group received maintenance medium. The latter group was included to determine whether there was an optimal time period for counting individual infected cells before the appearance of foci. Both test groups were incubated at 35 C for 4, 6, 8, 10, 12, 16, 21, or 25 hours. Representative cell monolayers from each group were then processed for fluorescent antibody staining.

The earliest sign of specific fluorescence of chikungunya virus antigens in cells was seen at 6 hours after infection. Fluorescence was localized exclusively in the cell cytoplasm and was confined to individual cells. At 8 hours, an occasional immunofluorescent focus that consisted of three or more infected cells was noted. Generally, the number of infected cells in a focus increased to 10 or more with prolonged incubation times (Fig. 3). Individual infected cells were mostly seen in cell monolayers incubated with maintenance medium; immunofluorescent foci were predominant in cell cultures incubated with antiviral serum. The increase in numbers of immunofluorescent foci and individual infected cells during incubation of the two test groups of cell monolayers is shown in Figure 4. In the presence of antiviral serum, number of immunofluorescent foci increased during the first 12 hours of incubation and thereafter remained constant. Individual infected cell counts in cell monolayers incubated with maintenance medium increased almost linearly throughout the 25-hour incubation period, which made it difficult to delineate the primary from secondary cycle of infection. In view of these findings, the assay of chikungunya virus was based on immunofluorescent focus counting. Foci could be enumerated as early as 16 hours after inoculation of cell monolayers to obtain an estimate of virus titer.

E. QUANTITATIVE EVALUATIONS OF THE ASSAY

A linear relationship was obtained between the number of immunofluorescent focus units and relative virus concentration throughout the inoculum range of 1.2 $\log_{10}$ units (Fig. 5). Each immunofluorescent focus appeared to result from infection by a single infective virus particle or aggregate not divisible by dilution.

FIGURE 3. Immunofluorescent Focus of Chikungunya Virus Infection in BHK21/13 Cells in the Presence of Antiviral Serum. 225X.

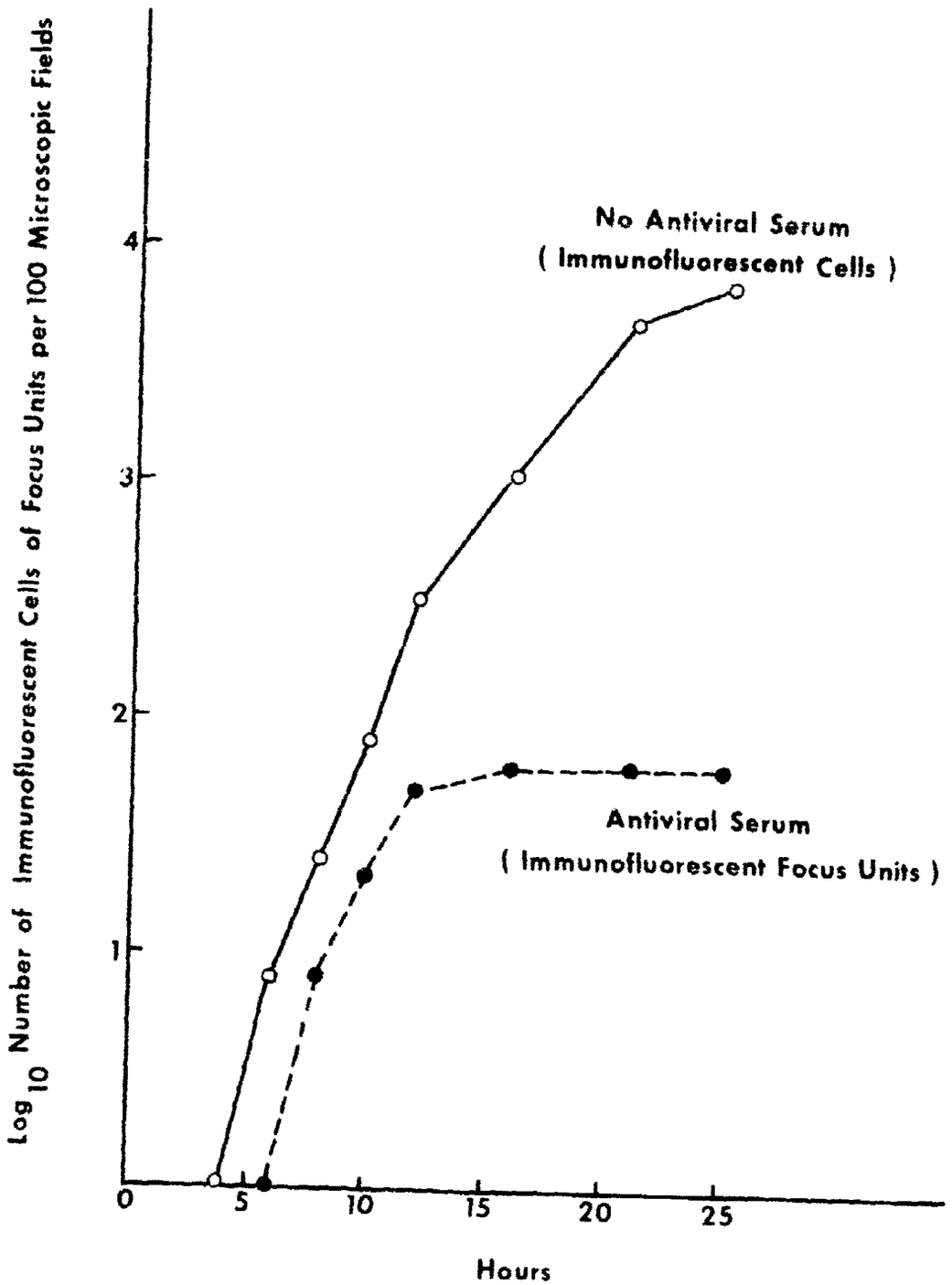

FIGURE 4. Effect of Length of Incubation Period (35 C) on Number of Infected BHK21/C13 Cells in the Presence and Absence of Antiviral Serum.

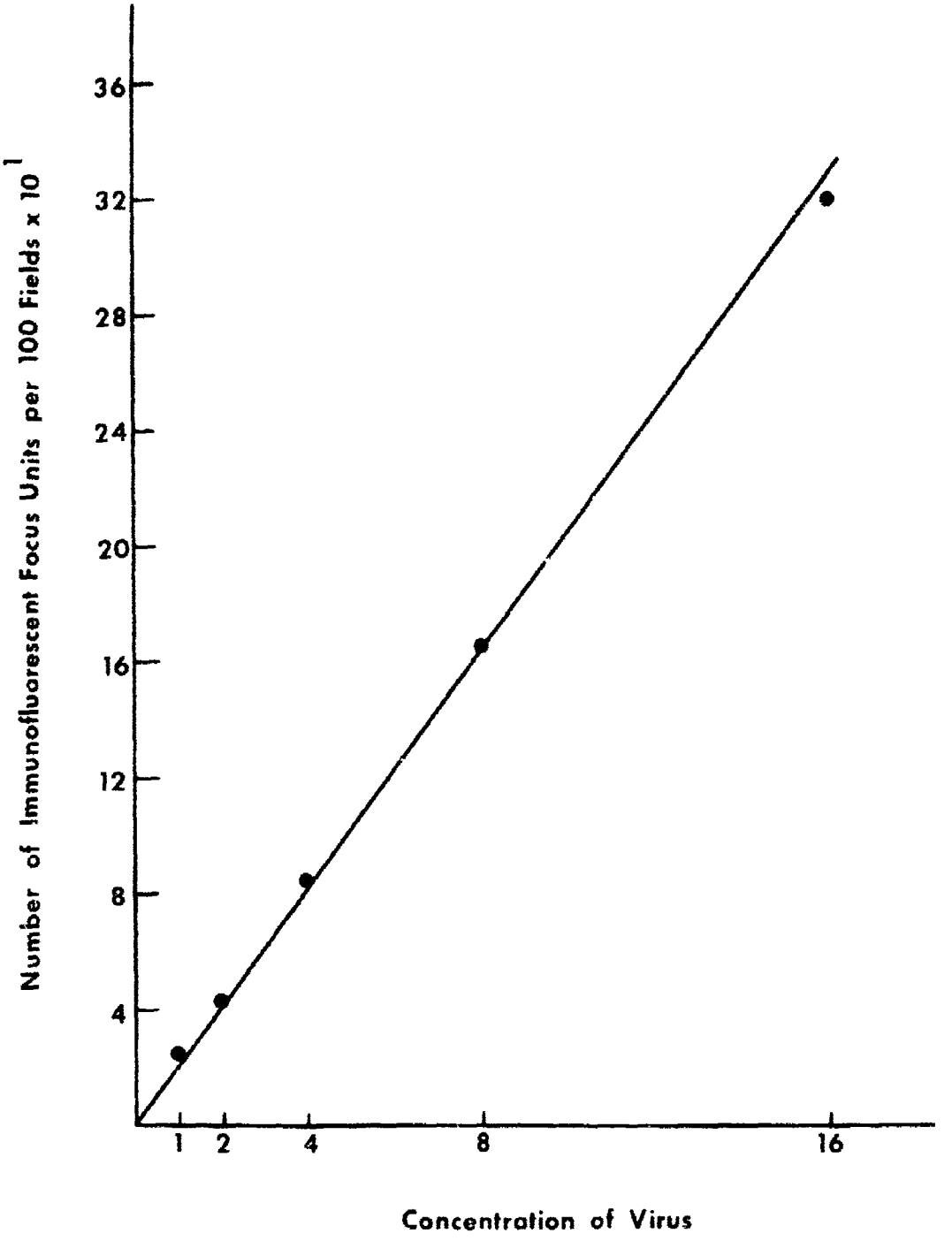

FIGURE 5. Linear Function Between the Number of Immunofluorescent Focus Units and Relative Concentration of Chikungunya Virus.

Ten determinations were performed in a single experiment to estimate the precision of the assay. Cover slip cell monolayers were infected with 0.2 ml of a 10^{-5} virus dilution and then treated in the prescribed manner. The number of IFU per milliliter of virus inoculum ranged from 5.2×10^8 to 6.3×10^8 with a mean of 5.8×10^8, standard deviation of ±0.17, and coefficient of variation of 2.9%.

To determine the reproducibility of the immunofluorescent focus counting assay, a viral of virus was randomly selected at approximately 2-week intervals from the standard pool stored at -60 C and assayed. In four such trials, assay values ranged from 5.2×10^8 to 6.3×10^8 IFU/ml with a mean of 5.8×10^8.

The sensitivity of the immunofluorescent focus counting assay of chikungunya virus was compared with that of the methods of intracerebral inoculation of suckling mice and virus plaque counting. CEF, GPL, and BHK21/C13 cell monolayers were each employed for virus assay by the plaque procedure. Six determinations were made with each assay using virus from a standard pool. Virus assays in either suckling mice or by counting virus-induced plaques in BHK21/C13 cell monolayers appeared to be comparable in sensitivity (Table 4). The mean assay value obtained by immunofluorescent focus counting was only 0.2 $\log_{10}$ unit less than that obtained by the former procedures. The mean assay value derived from plaque counts using GPL cell monolayers was 0.2 $\log_{10}$ unit less than that obtained by the immunofluorescent focus counting method. In CEF cell monolayers, plaques were not formed with the virus dilutions inoculated. The immunofluorescent focus counting assay, however, exhibited less variability than that of the other virus assay methods tested and, in addition, had the singular advantage of rapidity (less than 24 hours).

F. SERUM NEUTRALIZATION TEST

A preliminary test was made to determine whether the immunofluorescent focus counting assay of chikungunya virus could be adapted for estimating serum-neutralizing antibodies against the virus. Appropriate dilutions of monkey antiviral or normal serum (control) were mixed with equal volumes of a constant quantity of virus (5.0×10^4 IFU/ml). Virus and serum dilutions were prepared in PBS. After test mixtures were incubated at 35 C for 2 hours, 0.2 ml of each mixture was introduced onto one of three cover slip BHK21/C13 cell monolayers for assay of residual virus infectivity in the manner described previously. To determine the 50% serum-neutralizing end point, the percentage reduction of immunofluorescent focus counts for each antiviral serum dilution was computed from the control counts. Reduction percentages were then plotted against the logarithm of the corresponding final dilutions of antiviral serum on probability paper. Results in Figure 6 show that a linear relationship was obtained over a critical range. By interpolation, the dilution of antiviral serum that neutralized 50% of virus was determined.

TABLE 4. ASSAY OF CHIKUNGUNYA VIRUS BY DIFFERENT METHODS

Assay No.	IFU[a]/ml, x 10^8	SMICLD$_{50}$[b]/ml, x 10^8	PFU[c]/ml, x 10^8	
			BHK21/C13 Cells	GPL Cells
1	5.3	9.5	7.6	3.6
2	5.8	10.0	7.0	3.2
3	5.3	12.0	7.6	3.6
4	5.1	0.63	8.8	2.0
5	5.1	10.0	10.0	3.6
6	4.8	10.0	7.8	4.7
Mean	5.2	8.6	8.1	3.4
Standard deviation	±0.34	±4.0	±1.1	±0.87
Standard error of mean	±0.13	±1.63	±0.45	±0.35

a. Immunofluorescent focus units of virus determined in 20 hours.
b. Suckling mouse intracerebral median lethal units of virus determined in 10 days.
c. Plaque-forming units of virus in BHK21/C13 and guinea pig lung cell monolayers determined at 3 and 4 days, respectively.

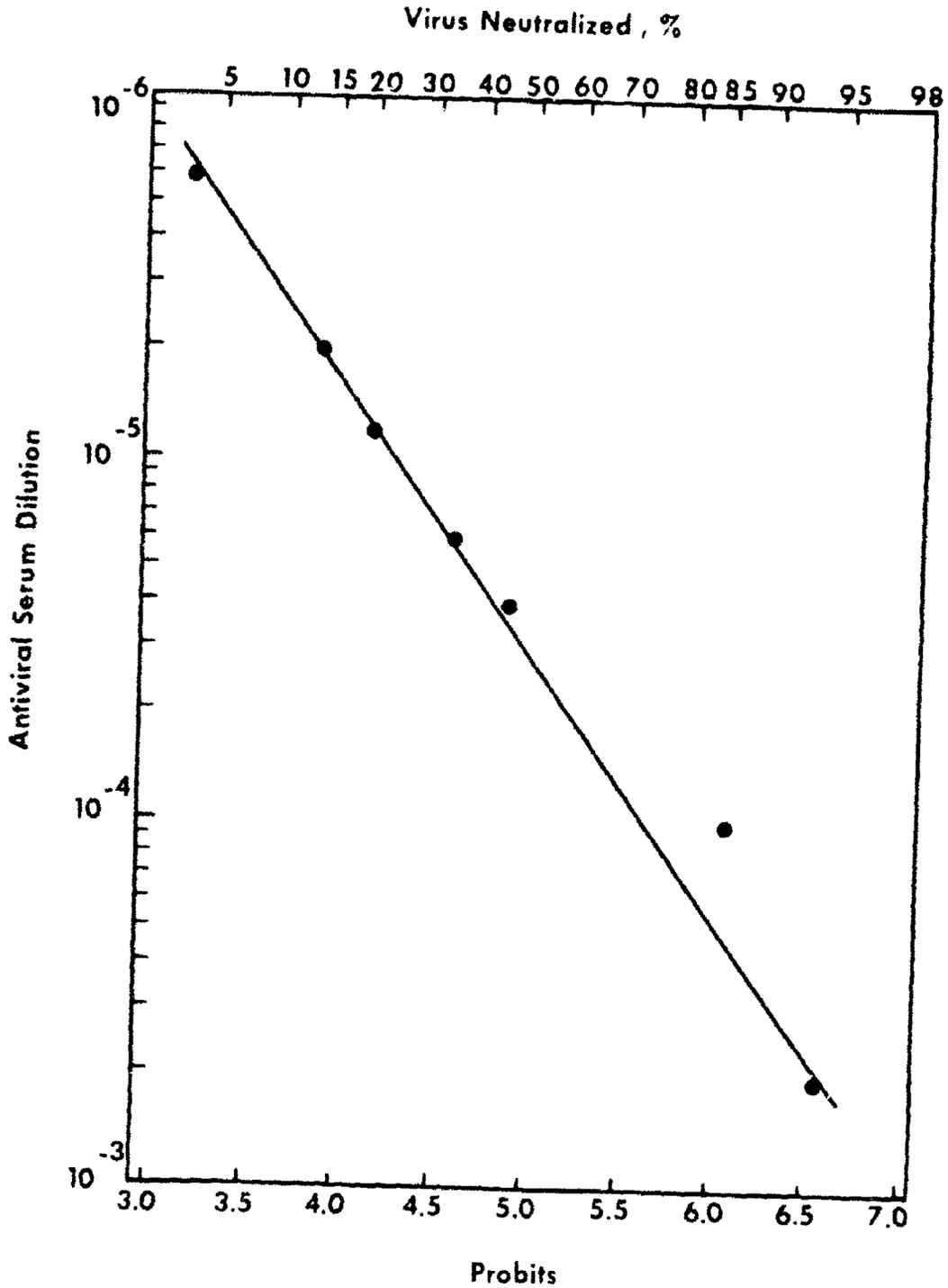

FIGURE 6. Estimation of Chikungunya Serum-Neutralizing Antibodies by the Immunofluorescent Virus Assay Procedure. Fifty per cent serum-neutralizing end point was interpolated from the percentage reduction of immunofluorescent foci of infected cells by antiviral serum dilutions.

IV. DISCUSSION

The feasibility of employing the immunofluorescence procedure for the quantitative assessment of chikungunya virus infectivity was established by the studies described here. The assay was highly precise, reproducible, and comparable in sensitivity to other established methods of chikungunya virus assessment. The outstanding feature of the method, in common with immunofluorescent assays described for other arboviruses,[3,5] was the ability to assess virus infectivity within 24 hours after inoculation of cell monolayers. The time required to assay chikungunya virus by intracerebral injection of suckling mice or plaque counting was 10 days and from 3 to 4 days, respectively.

The use of centrifugal force for attaching virus onto cell monolayers offers several advantages over stationary incubation. By the former procedure, virus attachment is efficient and rapid, thermal inactivation of virus inoculum is minimized, and the proportionality demonstrated between immunofluorescent focus counts and volume of inoculum facilitates the detection of virus particles from dilute suspensions. With the use of centrifugal force, almost synchronous infection of cells may be achieved and the stages of virus attachment onto and penetration into cells may be readily delineated. These are highly desirable advantages in studies concerned with the early stages of virus replication.

For attachment of chikungunya virus to cells, the requirements were less demanding than those for VEE virus. Maximal attachment of chikungunya virus occurred in the presence of menstruums of varied composition. With VEE virus, however, maximal attachment to cells occurred only in the presence of PBS free of calcium and magnesium ions. In contrast to the findings with chikungunya virus, HIB markedly inhibited VEE virus attachment.[3] This indicates that there is an inherent difference in the requirements of these two group A arboviruses for effecting maximal attachment to cells.

An attempt to base the assay of chikungunya virus on counts of individual infected cells was hampered by the appearance of foci of infected cells in BHK21/C13 cell monolayers. These foci occurred even when inoculated cell monolayers were incubated in the presence of a potent antiviral serum overlay. When different established cell lines were inoculated and incubated with antiviral serum, virus infection was confined to individual cells. In earlier studies, direct evidence was obtained to show that chikungunya virus infection of BHK21/C13 cell monolayers occurs by cell-to-cell transmission in the presence of antiviral serum overlay and, also, extracellularly in the absence of the overlay.* Under similar circumstances, foci of VEE virus - infected cells in BHK21/C13 cell cultures also occurred in the presence of antiviral serum, but infection was limited to individual cells

* Hahon and Zimmerman, in preparation.

when different cell lines were used. These observations suggest that BHK21 cell lines possess some unique biological membrane structure or physiology that is conducive for cell-to-cell transmission of virus particles. It may be of some relevance that the cell line has been extensively employed for neoplastic transformation by viruses.[13] Because BHK21/C13 cells were the most susceptible of the cell lines tested to chikungunya virus infection, it was the host cell line selected for assay of the virus. The assay was based, therefore, on the appearance of immunofluorescent foci that could be enumerated as early as 16 hours after inoculation of cell monolayers.

In a preliminary test, the 50% neutralizing end point of antiviral serum was determined by reacting chikungunya virus with antiserum. Over a critical range, a linear relationship was obtained between reduction percentages of immunofluorescent focus counts and dilutions of antiviral serum. This finding augurs well for the feasibility of estimating, rapidly and quantitatively, chikungunya serum-neutralizing antibodies by the immunofluorescence virus assay procedure.

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13. ABSTRACT

Chikungunya virus was assayed quantitatively by counting immunofluorescent foci after infection of BHK21/C13 cell monolayers. The speed and efficiency of virus attachment to cells were markedly enhanced when augmented by centrifugal force. By this procedure, a proportionality was obtained between the number of immunofluorescent foci and the volume of inoculum. Virus penetration into cells was linear and complete within 15 minutes at 35 C. From observations on the sequential development of viral antigen within cells and immunofluorescent focus counts, foci of infected cells may be enumerated as early as 16 hours after inoculation of cell monolayers. A linear function was demonstrated between immunofluorescent focus counts and relative virus concentration. The immunofluorescent assay was comparable in sensitivity to but more precise and rapid than virus assays based on the intracerebral inoculation of suckling mice or on plaque counting. By the immunofluorescence procedure, the 50% neutralizing end point of antiviral serum was rapidly and quantitatively determined.

14. Key Words

Chikungunya virus
Assay
Immunofluorescent focus counting
Serum neutralization

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